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2(*S*),4(*R*)-4-(β-D-GALACTOPYRANOSYLOXY)-4-ISOBUTYLGLUTAMIC ACID: A NEW AMINO ACID IN *RESEDA ODORATA*

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Key Word Index—*Reseda odorata*; Resedaceae; non-protein amino acid; *O*-galactoside; galactosyloxyamino acid; 2(*S*),4(*R*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid; 4-hydroxy-4-isobutylglutamic acid; ^{13}C-NMR spectroscopy.

Abstract—2(*S*),4(*R*)-4-(β-D-Galactopyranosyloxy)-4-isobutylglutamic acid (I) has been isolated from the flowers of *Reseda odorata*, wherein it occurs in substantial quantity. Hydrolysis of I gives D-galactose, 2(*S*),4(*R*)-4-hydroxy-4-isobutylglutamic acid (II) and 3(*R*),5(*S*)-3-hydroxy-3-isobutyl-2-pyrrolidone-5-carboxylic acid (III) and its treatment with nitrous acid yields a galactoside of a non-nitrogenous hydroxy acid lactone (IV). The structures of I and its degradation products are supported by PMR, ^{13}C-NMR and other spectroscopic methods. ^{13}C-NMR spectroscopy of the model compound 2-(β-D-galactopyranosyloxy)isobutyric acid confirmed the structure of the natural product. The *S*- (or L-) configuration at C(2) in the amino acid moiety of I has been established by the use of the Clough–Lutz–Jirgenson rule and the *R*-configuration at C(4) of the same unit has been assigned tentatively. I represents the first example of a glycoside of a higher plant amino acid in which the carbohydrate residue is linked to an aliphatic hydroxy group.

INTRODUCTION

THE PRESENT work is a continuation of previous investigations on the free amino acids and amines in the Resedaceae and Cruciferae.[1,2] Studies on flowers of *Reseda odorata* L. revealed the presence of a new amino acid, as one of the major ninhydrin-reacting compounds. This amino acid now has been isolated in substantial quantity. By chemical and spectroscopic studies of it and its degradation products, it has been identified as 2(*S*),4(*R*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid (I). A brief account of part of this work has been given previously.[3]

RESULTS

Compound I has been found only in flowers and in no other parts of the plant. Its concentration appears to depend on the illumination, reaching the level of glutamic acid, glycine, and valine on sunny days but falling to negligible values in dull weather. I was

[1] SØRENSEN, H. (1970) *Phytochemistry* **9**, 865.
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[3] LARSEN, P. O., SØRENSEN, H. and SØRUP, P. (1972) *IV Internat. Symp. Biochem. u. Physiol. d. Alkaloide.* Halle, 1969, Abh. Dtsch. Akad. Wiss. *Symposiumsbericht Band* 6 (MOTHES, K. and SCHÜTTE, H. R., eds.), p. 113, Akademiev, Berlin.

isolated by traditional methods, including ion-exchange chromatography and purification on Sephadex (see Experimental). From 1·2 kg flowers, 1·22 g of I was obtained as an amorphous colourless and chromatographically pure solid. Since the compound is heat- and acid-labile and hygroscopic, crystallization has not been possible.

Treatment of I with HCl transforms it in low yield to D-galactose, 2(*S*),4(*R*)-4-isobutyl-4-hydroxyglutamic acid (II), and 3(*R*),5(*S*)-3-hydroxy-3-isobutyl-2-pyrrolidone-5-carboxylic acid (III) and other, unidentified degradation products. An increase of temperature, time and HCl concentration in the hydrolysis leads to an increased yield of III, but a decrease in II and galactose.

Treatment of I with nitrous acid results in the production of the hydroxy acid lactone IV, reminiscent of the transformation of glutamic acid into 4-carboxy-4-butyrolactone.[4] Acid hydrolysis of IV resulted in the production of galactose.

TABLE 1. p*K* VALUES*

	I	II	V†[,5]	VI‡[5,6]	VII§[,7]	VIII‖[,7]
pK_1	2·2	1·7				
pK_2	2·9	3·5	4·2	3·6	4·3	3·6
pK_3	10·5	9·7	9·7	9·7		

* Determined by potentiometric titration.
† Glutamic acid.
‡ 2(*S*),4(*R*)-4-Hydroxyglutamic acid.
§ 2(*S*),4(*S*)-4-Hydroxy-4-methylglutamic acid.
‖ 2(*S*),4(*R*)-4-Hydroxy-4-methylglutamic acid.

The acid character of I to IV is revealed by paper electrophoresis as well as by the behaviour of the compounds on ion-exchange resins. The p*K*s of I, II and reference compounds are listed in Table 1. The pK_2s are in accord with the elution behaviour of the substances on strongly basic ion-exchange resins.[8,9] The pK_3 10·5 value of I is that expected for a normal alkylammonium ion rather than for an α-alkoxyalkylammonium species.[10] This fact, the relative stability of I to hydrolysis in contrast to glycosides of amines[11] and the transformation of I into IV on nitrous acid treatment indicate an *O*-galactosyl rather than *N*-galactosyl moiety in the natural product.

While amino acid II gives a normal purple ninhydrin colour, I reacts abnormally, yielding a yellow spot which becomes purple only in the course of hours (see Experimental). The ninhydrin reaction of II is masked by pretreatment of the paper with cupric nitrate, indicating that the amino group is in a α-position to a carboxyl group.[12] The R_fs of I, II and III are in agreement with the proposed structures.

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[6] VIRTANEN, A. I. (1957) *Festschr. Arthur Stoll,* p. 565.
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[10] INOUYE, S. (1968) *Chem. Pharm. Bull. (Tokyo)* **16,** 1134.
[11] WEITZEL, G., GEYER, H. U. and FRETZDORFF, A. M. (1957) *Chem. Ber.* **90,** 1153.
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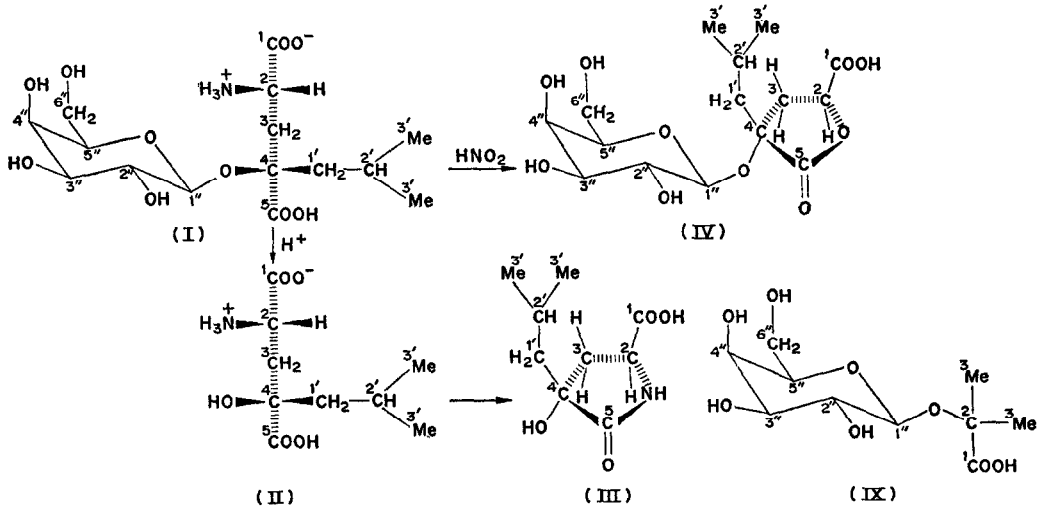
The MS of II and III have many peaks in common, since II is probably converted into III in the spectrometer. Predictably, no molecular ion peaks appear. Lactam III suffers fragmentation in the form of peaks at 184 (M − OH), 156 (M − CO_2H) and 145 (M − C_4H_8) (base peak). The fragmentation pattern of the MS of I varies with the inlet temperature. Peaks at 383 (M + 2H), 339 (M − CO_2), and 321 (M − (CO_2 + H_2O)) and those common to II and III appear.



Further structural information was obtained from ^{13}C and ^{1}H NMR spectroscopy.[13] The natural abundance noise resonance decoupled and single frequency off-resonance decoupled ^{13}C NMR spectra of I showed the presence of fifteen carbons in a glycosyl amino acid array. Comparison of the chemical shifts of the potential glycoside carbons with those recorded for methyl glycosides[14] establish the sugar residue as a β-galactosyl unit. The anomeric carbon shift indicates the presence of a tertiary alkoxy function at C(1″), as corroborated by the shift data of the synthetic 2-(β-D-galactopyranosyloxy)isobutyric acid (IX). The amino acid carbon shifts are in full agreement with the structure of a 4-hydroxy-4-isobutylglutamic acid moiety. Differentiation of the carboxyl and methylene carbon pairs is based on a pH study. Comparison of the spectrum of I at pH 0·5 with that at pH 11·0 gives $\Delta\delta$ values ($\Delta\delta = \delta^{pH\ 0\cdot5} - \delta^{pH\ 11\cdot0}$) of −10·9 and −4·3 ppm for the carboxyl carbons and −4·3 and 0·7 ppm for the methylene carbons. In view of known pH effects on the chemical shifts of carboxylic acids[15] and α-amino acids[16] these data leave the shift assignment of the glutamic acid residue unambiguous. The ^{13}C NMR results are summarized in Table 2.

Comparison of the PMR spectra of I and IV with those of α- and β-galactopyranosides[17]

[13] The ^{13}C NMR investigation represents contribution XVI in the series "^{13}C Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances". For the previous paper see LUKACS, G., PIRIOU, F., GERO, S. D., VAN DORP, D. A., HAGAMAN, E. W. and WENKERT, E. (1973) *Tetrahedron Letters* 515.

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[17] LEMIEUX, R. U. and STEVENS, J. D. (1966) *Can. J. Chem.* **44**, 249.

TABLE 2. ^{13}C NMR CHEMICAL SHIFTS*

	I	IX	X†	XI†
C (1)	172·6	178·1		
C (2)	51·3	78·4		
C (3)	38·8	23·8, 23·5		
C (4)	83·3			
C (5)	176·3			
C (1′)	42·9			
C (2′)	23·6			
C (3′)	21·9, 21·4			
C (1″)	97·7	97·7	103·7	99·4
C (2″)	70·6	70·6	70·8	69·5
C (3″)	72·1	72·6	72·9	69·8
C (4″)	68·2	68·3	68·7	68·5
C (5″)	74·8	74·7	74·9	70·8
C (6″)	60·4	60·4	61·0	61·4

* Spectra of aqueous solutions of 2(*S*),4(*R*)-4-(β-D-galactopyranosyloxy)-4-isobutylglutamic acid (I) and 2-(β-D-galactopyranosyloxy)isobutyric acid (IX) taken at 15·08 MHz on a Fourier transform spectrometer; chemical shifts in ppm downfield from TMS; $\delta^{TMS} = \delta^{dioxane} + 66{\cdot}3$ ppm (dioxane used as internal standard). For designation of atoms see formula chart.

† Values of methyl β-D-galactopyranoside (X) and methyl α-D-galactopyranoside (XI) from Ref. 14.

shows these substances to possess an *O*-β-galactopyranosyl unit (see Experimental). Table 3 lists the chemical shifts and coupling constants of the hydrogens of the amino acid portion of I–IV and reference compounds. These combined PMR data are in agreement with structure I for the natural product.

TABLE 3. ^1H NMR CHEMICAL SHIFTS AND COUPLING CONSTANTS*

	I†	II†	III	IV	VII‡	VIII‡
H (2)	4·3 (*J* 9·2, 3·7)	3·4 (*J* 11·1, 1·9)	4·5 (*J* 6·5)	5·1 (*J* 6·5)	3·6 (*J* 7·2, 5·7)	3·7 (*J* 9·5, 3·3)
H (3)	2·8, 2·5 (*J* −15·0)	2·3, 1·8 (*J* −14·5)	2·8	2·8	2·3, 2·0 (*J* −14·3)	2·5, 2·0 (*J* −15·0)
H (1′), H (2′)	1·5–2·0 (m)	1·3–1·8 (m)	1·5–2·4 (m)	1·5–2·0 (m)		
H (3′)	0·85, 0·85 (*J* 5·5)	0·8–0·9 (*J* 5·5)	1·0–1·1 (*J* 6)	0·85, 0·85 (*J* 5·5)		

* Spectra of D_2O solutions of I and IV, of an ammoniacal D_2O solution of II, and of a pyridine-D_5 solution of III taken on a JEOL C-60HL spectrometer; chemical shifts in ppm downfield from TMS in pyridine-D_5 and from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate in D_2O; *J* values in Hz; For designation of atoms see formula chart.

† *J* and δ values refined by spectral simulation.

‡ Spectra of D_2O solutions containing NaOD of 2(*S*),4(*S*)-4-hydroxy-4-methylglutamic acid (VII) and 2(*S*),4(*R*)-4-hydroxy-4-methylglutamic acid (VIII), from Ref. 18.

[18] ALDERWEIRELDT, F., JADOT, J., CASIMIR, J. and LOFFET, A. (1967) *Biochim. Biophys. Acta* **136**, 89.

The molecular rotations of II in water and HCl are −29·5° and +5·3° at the D-line, respectively. According to the Clough–Lutz–Jirgenson rule[19,20] this indicates a *S*- (or L-) configuration at the amino acid center, if the rotatory contribution of the (C4) center is assumed not to be influenced by the change of solvents from water to acid.[21] This assumption is justified, as shown by the literature values for C(2) and C(4) contributions in 4-hydroxyglutamic, 4-methylglutamic and 4-hydroxy-4-methylglutamic acids (Table 4). On the basis of the similarity of the p*K* and ^{1}H NMR properties of I (Tables 1 and 3) with those of 2(*S*),4(*R*)-4-hydroxy-4-methylglutamic acid (VIII) the natural substance can be assigned tentatively a *R* configuration at C(4). Completion of the synthesis of the two diastereomers of II now in progress should permit a definite assignment.

TABLE 4. M_D VALUES

	XII*	VI†	VII‡	VIII§	III‖	XIV¶
Water	+31·8°	−22·3° (+5°, −27°)**,9	−59·1°	+1·0° (−29°, +30°)**,18	−1·3°	−8·2° (−5°, −4°)**,22
Aq. HCl	+61·6°	+5·0° (+33°, −28°)**,9	−16·2°	+45·2° (+15°, +31°)**,18	+35·7	+30·9° (+33°, −2°)**,22

* 2(*S*),4(*S*)-4-Hydroxyglutamic acid.
† 2(*S*),4(*R*)-4-Hydroxyglutamic acid.
‡ 2(*S*),4(*S*)-4-Hydroxy-4-methylglutamic acid.
§ 2(*S*),4(*R*)-4-Hydroxy-4-methylglutamic acid.
‖ 2(*S*),4(*S*)-4-Methylglutamic acid.
¶ 2(*S*),4(*R*)-4-Methylglutamic acid.
** Contributions from the 2(*S*) and 4(*R*) centers, respectively.

DISCUSSION

Numerous 4-substituted glutamic acid derivatives have been identified in higher plants, e.g. 4-hydroxyglutamic, 4-methylglutamic, 4-hydroxy-4-methylglutamic, 4-methyleneglutamic, 4-ethylideneglutamic, 3-hydroxy-4-methylglutamic, and 3,4-dihydroxyglutamic acids.[7,12,18,22–25] All have a C(2) L- (*S*-) configuration. Both *R* and *S* configuration at C(4) occur, sometimes even in the same plant.[18,25]

The co-occurrence of several of the amino acids in the same plants has suggested a common biogenetic origin.[23] The possibility of the keto acid corresponding to 4-hydroxy-4-methylglutamic acid being derived from two pyruvic acid units has been made unlikely by recent investigations.[23,25] As a consequence the possible derivation of the keto acid corresponding to II from a condensation of pyruvic acid and 2-keto-4-methylpentanoic acid, the keto acid of leucine, appears unattractive at this time.

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[25] DARDENNE, G. A., BELL, E. A., NULU, J. R. and CONE, C. (1972) *Phytochemistry* **11,** 791.

Whereas various phenyl glucosides of aromatic and heteroaromatic amino acids have been found in higher plants recently,[3,26–28] I is the first example of a glycoside of an amino acid in which the carbohydrate is linked to an aliphatic hydroxy group. Glycosides of 5-hydroxylysine are known from microorganisms[29] and animals.[30]

EXPERIMENTAL

Isolation of I. Plants of *Reseda odorata* L. were grown from seeds of garden hybride material designated 'grandiflora' purchased from I. E. Ohlsen's Enke, Copenhagen. Freshly harvested flowers were homogenized in CCl_4 and defatted 2× with CCl_4 by refluxing (2·5 hr), cooling, and filtering. After being dried in the air the residue (1·2 kg) was extracted 2× with MeOH–H_2O (7:3, 5·5 l. each time) by refluxing (4 hr), cooling, and filtering. The combined filtrates were concentrated to dryness (290 g). The residue was suspended in H_2O (1·2 l.), filtered and applied to a strongly acid ion-exchange resin (Amberlite IR 120, H^+, 6·5 × 70 cm). The latter was washed with H_2O (25 l.) and the amino acids were eluted with aq. ammonia (2 N, 7 l.). The eluate was evaporated to dryness (59 g), and the residue suspended in H_2O (500 ml) and filtered. The filtrate was applied to a strongly basic ion-exchange resin (Dowex 1 × 4, AcO^-, 20–50 mesh, 3 × 90 cm) and the latter washed with H_2O (5 l.) and eluted with 1M HOAc). Fractions of 15 ml each were collected, leading to the isolation of I in fractions 206–335 after the fractions with aspartic and glutamic acids. Evaporation of the eluates containing I to dryness gave 2·5 g of a yellow, amorphous residue. Further purification was accomplished by passage through a strongly basic resin (Dowex 1 × 8, AcO^-, 200–400 mesh, 1·5 × 90 cm, elution of I with 1 M HOAc, yield 1·5 g), a strongly acid resin (Dowex 50W × 8, H^+, 200–400 mesh, 0·9 × 60 cm, elution of I with 1 M pyridine, yield 1·46 g), a strongly basic resin (Dowex 1 × 8, AcO^-, 200–400 mesh, 0·9 × 60 cm, elution with 1 M HOAc, yield 1·40 g), and, finally, a Sephadex G10 column (2·5 × 45 cm, elution with H_2O, 40 ml/hr, 10 ml fractions). Fractions 17–23 yielded pure I (1·22 g) as a colourless amorphous solid $[\alpha]_D^{25}$ −1·6° (*c* 2, H_2O). PMR spectroscopy demonstrated the presence of H(1″) (the anomer proton) at 4·6 ppm (*J* 6·5 Hz) indicating β-configuration.[17] The five remaining protons in the carbohydrate moiety occurred as a complex pattern at 3·3–4·2 ppm. For the protons in the amino acid moiety see Table 3 (Found: C, 43·63; H, 7·37; N, 3·05; H_2O, 7·0. $C_{15}H_{27}NO_{10}$, $2H_2O$ required: C, 43·16; H, 7·49; N, 3·36; H_2O, 8·63%).

Hydrolysis of I. (A). A solution of I (230 mg) in HCl (5 ml, 1 N) was refluxed (5 hr) and evaporated to dryness. After 3 successive dissolutions and evaporations each from H_2O and acetone the dark, semisolid residue was dissolved in H_2O and applied to Dowex 50W × 8 resin (H^+, 200–400 mesh, 0·5 × 5 cm). After being washed with H_2O (5 ml) the column was eluted with aq. pyridine (1 M, 3 ml). The pyridine eluate was concentrated to dryness (74 mg), dissolved in H_2O, and applied to Dowex 1 × 8 resin (AcO^-, 200–400 mesh, 0·5 × 5 cm). After being washed with H_2O (5 ml) the column was eluted with 10 ml 1 M HOAc. The eluate was evaporated to dryness (53 mg). Three crystallizations of the residue from H_2O afforded II as a colourless crystalline solid (17 mg), $[\alpha]_D^{25}$ −13° (*c* 0·4, H_2O), $[\alpha]_D^{25}$ +2° (*c* 0·2, 6 N HCl). IR: ν_{max}^{KBr} 2960 cm^{-1} (strong), 2930 (medium), 2860 (s), 1740 (s), 1720 (s), 1640 (s), 1630 (s), 1595 (s), 1540 (s), 1490 (s), 1405 (s), 1365 (weak), 1340 (w), 1325 (s), 1270 (s). (Found: C, 46·96; H, 7·96; N, 5·75; H_2O, 6·2. $C_9H_{17}NO_5 \cdot 3/4H_2O$ required: C, 46·44; H, 8·01; N, 5·75; H_2O, 5·81%). The H_2O eluate from the acid ion-exchange resin was evaporated to dryness (152 mg). The residue was dissolved in H_2O and applied to a strongly basic ion-exchange resin as above. The eluate of this column yielded only a trace of galactose. After concentration of the HOAc eluate to dryness (74 mg) and two crystallizations of the residue from ether III was obtained (22 mg), $[\alpha]_D^{18}$ −11·5° (*c* 0·1, H_2O). IR: ν_{max}^{KBr} 2960 cm^{-1} (s), 2930 (m), 2880 (s), 1740 (s), 1720 (s), 1700 (s), 1660 (s), 1450 (m), 1420 (s), 1390 (s), 1365 (w), 1340 (w), 1320 (w), 1300 (s) (Found: C, 54·0; H, 7·5; N, 6·9. $C_9H_{15}NO_4$ required: C, 53·72; H, 7·51; N, 6·96%).

Hydrolysis of I (B). A solution of I (215 mg) in HCl (5 ml, 1 N) was heated to 90° for 30 min and evaporated to dryness. The residue was extracted with Et_2O (3 × 30 ml), dissolved in H_2O, and applied to a strongly basic ion-exchange resin (Dowex 1 × 8, AcO^-, 200–400 mesh, 0·9 × 60 cm). Elution with 0·5 M HOAc and evaporation of the first 400 ml of the eluate to dryness (90 mg) gave a residue which was dissolved in H_2O and passed through a strongly acid ion-exchange resin (Dowex 50W × 8, H^+, 200–400 mesh, 0·7 × 10 cm). Evaporation of the eluate to dryness gave a semisolid residue of galactose (45·5 mg) which was purified

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[31] WOLFROM, M. L. and THOMPSON, A. (1962) *Methods Carbohydr. Chem.* **1,** 121.

by preparative PC (Whatman No. 3MM, solvent 1, see below) and Sephadex G10 column chromatography (2·5 × 45 cm). The latter was eluted with H_2O (40 ml/hr), 10 ml fractions being collected. Evaporation of fractions 20–22 yielded crystalline D-galactose (11 mg), $[\alpha]_D^{25}$ +80° (*c* 0·4, H_2O) (lit.[31] $[\alpha]_D^{20}$ +80° (*c* 5, H_2O)); IR and PMR spectral and PC properties identical with those of an authentic material. In this hydrolysis 15 mg of III were isolated from the Et_2O extracts and 37 mg of unreacted I and 25 mg of II from the acetic acid eluate of the basic ion-exchange resin.

Treatment of I *with nitrous acid.* A solution of I (70 mg) and $NaNO_2$ (0·3 mmol) in D_2O (0·5 ml) was kept at room temp. until cessation of N_2 evolution and constancy of the NMR spectrum was reached (2 hr). PC investigation of the reaction mixture demonstrated the presence of IV, identified by its R_f in solvent 1 (see below) and its positive Rydon-Smith[32] and periodate[33] tests, and a trace of I. The solution was applied to a strongly basic ion-exchange resin (Dowex 1 × 8, AcO^-, 200–400 mesh, 0·5 × 3 cm). The column was washed with H_2O (25 ml) and 30 ml 2M HOAc and eluted with formic acid (1 M, 10 ml). Evaporation of the eluate yielded IV (42 mg) as a semicrystalline residue, IR. ν_{max}^{KBr} 1785 cm^{-1} (strong), 1620 (s). PMR spectroscopy demonstrated the presence of H(1″) at 4·6 ppm (*J* = 6·5 Hz), the five remaining protons in the carbohydrate moiety occurring as a complex pattern at 3·3–4·2 ppm. For the protons in the carboxylic acid moiety see Table 3 (Found: N, 0·8. $C_{15}H_{24}O_9$ requires: N, 0%). Hydrolysis of IV in HCl (1 N) at 100° for 1 hr yielded galactose, identified by PC.

PC. PC was performed in *n*-BuOH–HOAc–H_2O (12:3:5) (Solvent 1), PhOH–H_2O–conc. NH_3 (120:30:1) (w/v/v) (Solvent 2) and *iso*PrOH–conc. NH_3–H_2O (8:1:1) (v/v/v) (Solvent 3). The following R_fs were found, by descent, on Whatman No. 1: Compound I: 0·30, 0·35, 0·15 in solvents 1, 2 and 3 respectively; Compound II: 0·55, 0·50, 0·25; Compound III: 0·80, 0·70 in solvents 1 and 2 respectively. Compound I reacts abnormally with ninhydrin at room temp. and at 110°, yielding a yellow spot which becomes purple only in the course of hours. Heating of the PCs with ninhydrin to 50° for 1 min results, however, in the immediate production of a normal purple spot. Compound III was detected on PCs with the Rydon-Smith reagent.[32]

Synthesis of 2-(*β*-D-*galactopyranosyloxy*)*isobutyric acid* (IX). A mixture of ethyl 2-hydroxy-2-methylpropionate (70 mmol), tetra-*O*-acetyl-α-D-galactopyranosyl bromide[34,35] (80 mmol) and Ac_2O (80 mmol) in C_6H_6 (300 ml) was stirred for 2 hr at room temp., filtered and evaporated to dryness (40 g) (compare[36,37]). Ethyl 2-(tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)-2-methylpropionate was isolated (13·5 g) from the residue by chromatography on a silica gel column (3·5 × 100 cm) with C_6H_6–acetone (1:1) as mobile phase. The ester was hydrolyzed in 0·5 l. 0·1 M $Ba(OH)_2$ at 30° for 48 hr. After saturation of the hydrolysis mixture with CO_2, removal of $BaCO_3$ by centrifugation, and evaporation to dryness, IX was isolated by chromatography on a strongly basic ion-exchange resin (Dowex 1 × 8, AcO^-, 200–400 mesh, 0·9 × 60 cm, elution with HOAC). PMR spectroscopy showed the anomeric proton at 4·65 ppm (*J* 6·5 Hz), the six protons in the two methyl groups as a singlet at 1·45 ppm and the remaining protons from the carbohydrate moiety at 3·2–4·2 ppm. For ^{13}C NMR spectroscopic data see Table 2.

General methods and instrumentation. IR spectra were determined in KBr pellets. Optical rotations were determined on a Perkin Elmer Model 141 photoelectric polarimeter in 1 dm tubes. Microanalyses were performed by Mr. G. Cornali, Copenhagen. The analysis of III was performed as an ultramicroanalysis by Dr. W. Kirsten, Uppsala, Sweden. Water contents was determined as wt loss when the samples were dried over P_2O_5. The water was taken up again when the samples were exposed to the atmosphere. MS were determined on an AEI Model MS902 instrument at the H. C. Ørsted Institute, University of Copenhagen.

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